Laboratory Procedure Manual

Analyte:	Sulfonylurea Herbicides
Matrix:	Urine
Method:	HPLC-MS/MS
Method No.:	
Revised:	
as performed by:	*Toxicology Branch* *Division of Laboratory Sciences* *National Center for Environmental Health, CDC*
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Important Information for Users
CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Sulfonylurea Herbicides in Urine
NHANES 2007-2008
Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label (and SI units)
UPP_E	URXBSM	Bensulfuron-methyl (µg/L)
	URXCHS	Chlorsulfuron (µg/L)
	URXEMM	Ethametsulfuron methyl(µg/L)
	URXFRM	Foramsulfuron (µg/L)
	URXHLS	Halosulfuron methyl(µg/L)
	URXMTM	Metsulfuron methyl (µg/L)
	URXMSM	Mesosulfuron methyl(µg/L)
	URXNOS	Nicosulfuron(µg/L)
	URXOXS	Oxasulfuron(µg/L)
	URXPIM	Primisulfuron methyl (µg/L)
	URXPRO	Prosulfuron (µg/L)
	URXRIM	Rimsulfuron (µg/L)
	URXSMM	Sulfometuron methyl (µg/L)
	URXSSF	Sulfosulfuron (µg/L)
	URXTRA	Triasulfuron (µg/L)
	URXTHF	Thifensulfuron methyl (µg/L)
	URXTRN	Triflusulfuron methyl (µg/L)
	URXUCR	Creatinine, urine (mg/mL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

A method for measuring 17 sulfonylurea (SU) herbicides in human urine was developed. Urine samples were extracted using solid phase extraction (SPE), pre-concentrated, and analyzed by high-performance liquid chromatography–tandem mass spectrometry using turbo-ionspray atmospheric pressure ionization. Carbon 13-labeled ethametsulfuron methyl was used as an internal standard. Chromatographic retention times were under 7 minutes. Total throughput was estimated as >100 samples per day. The limits of detection (LOD) ranged from 0.05 µg/L to 0.10 µg/L with an average LOD of 0.06 µg/L. Average total relative standard deviations were 17%, 12% and 8% at 0.1 µg/L, 3.0 µg/L and 10 µg/L, respectively. Average extraction efficiencies of the SPE cartridges were 87% and 86% at 2.5 µg/L and 25 µg/L, respectively. Chemical degradation in acetonitrile and urine was monitored over 250 days. Estimated days for 10% and 50% degradation in urine and acetonitrile ranged from 0.7 days to >318 days.

Because of their increasing use in agricultural applications, a method to measure both occupational and incidental human exposures to SU herbicides was developed,

2. SAFETY PRECAUTIONS

A. Reagent Toxicity or Carcinogenicity. Some of the reagents used are toxic. Special care should be taken to avoid inhalation or dermal exposure to the reagents necessary to carry out the procedure.

B. Radioactive Hazards. None

C. Microbiological Hazards. Although urine is generally regarded as less infectious than serum, the possibility of being exposed to various microbiological hazards exists. Appropriate measures should be taken to avoid any direct contact with the specimen (See Protective Equipment below). A Hepatitis B vaccination series is usually recommended for health care and laboratory workers who are exposed to human fluids and tissues.

D. Mechanical Hazards. There is only minimal mechanical hazard when performing this procedure using standard safety practices. Laboratorians should avoid any direct contact with the electronics of the mass spectrometer unless all power to the instrument is off. Generally, electronic maintenance and repair should only be performed by qualified technicians.

E Protective Equipment. Standard safety apparatus should be used when performing this procedure. This apparatus includes lab coat, safety glasses, durable gloves, and a chemical fume hood.

F. Training. Training and experience in the use of a triple quadrupole mass spectrometer should be obtained by anyone using this procedure. Formal training is not necessary; however, personnel should be trained appropriately by an experienced operator of the instrument and are required to read the operation manuals.

G. Personal Hygiene. Care should be taken in handling any biological specimen. Routine use of gloves and proper hand washing should be practiced.

H. Disposal of Wastes. Solvents and reagents should always be put to waste in an appropriate container clearly marked for waste products and temporarily stored in a flame resistant cabinet. Containers, glassware, etc., that come in direct contact with the specimens should be autoclaved or decontaminated with 10% bleach. The glassware should be washed and recycled or disposed of in an appropriate manner.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

A. Software and Knowledge Requirements. A database named NPP2 has been developed on the EHLS-PC Network using R: Base 4.5+ (Microrim Inc., Redmond, WA). This database is used for storage, retrieval, and analysis of data from the pesticide residue analyses. Statistical analyses of data are performed using Statistical Analysis System (SAS) software (SAS Institute, Cary, NC). Knowledge of and experience with these software packages (or their equivalent) are required to utilize and maintain the data management structure.

B. Sample Information. Information pertaining to particular specimens is transferred electronically into the database or manually entered. Data that are manually entered include the sample identification number, the

notebook number associated with the sample preparation, the sample type, standard number, and any other information not associated with the mass spectral analysis. The analytical information obtained from the sample is electronically transferred from a UNIX-based system to a PC via an ethernet connection. The data are then transferred electronically into the database.

C. Data Maintenance. All sample and analytical data are checked after being entered into the database for transcription errors and overall validity. The database is routinely (at least once weekly) backed up onto a computer hard drive and onto a network magnetic tape.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. Sample Collection. Urine specimens are collected from subjects in standard urine collection cups. Samples should be refrigerated as soon as possible and transferred to specimen vials within 4 hours of collection. A minimum of 20 milliliters of urine is collected, and poured into sterile 14mL vials with screw cap tops. The specimens are then labeled, frozen immediately to -20 °C, and stored on dry ice for shipping. Special care must be taken in packing to protect vials from breakage during shipment. All samples should be stored at -20 °C until analysis.

B. Sample Handling

Samples are thawed, aliquoted, and the residual specimen is again stored at -70 °C until needed.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

A. Chemicals

1. All solvents used were analytical grade with purity greater than 98%.

2. Ethyl ether was purchased from Tedia Company, INC. (Fairfield, Ohio).

3. Butyl chloride (BuCl) was purchased from Tedia Company, INC. (Fairfield, Ohio).

4. Methanol (MeOH) was purchased from Tedia Company, INC. (Fairfield, Ohio).

5. Hexane was purchased from Tedia Company, INC. (Fairfield, Ohio).

6. Acetonitrile was purchased from Tedia Company, INC. (Fairfield, Ohio).

7. Toluene was purchased from Tedia Company, INC. (Fairfield, Ohio).

8. Ascetic acid was purchased from J. T. Baker Co. (Phillipsburg, N.J.).

9. Sodium acetate was purchased from J. T. Baker Co. (Phillipsburg, N.J.).

10. Bensulfuron-methyl (99%), chlorsulfuron (98%), ethametsulfuron methyl (98%), halosulfuron methyl (99%), metsulfuron methyl (99%), primisulfuron methyl (99%), prosulfuron (98%), rimsulfuron (99%), sulfometuron methyl (98%), sulfosulfuron (98%), triasulfuron (98%), and triflusulfuron methyl (98%) were purchased from Chem Service (West Chester, PA).

11. Foramsulfuron (97%), mesosulfuron methyl (98%), nicosulfuron (97%), oxasulfuron (97%), and thifensulfuron methyl (98%) were purchased from EQ Laboratories (Augsburg, Germany).

12. Stable, isotopically labeled ($^{13}C_3$) - ethametsulfuron methyl (98%) was a generous gift from DuPont Corporation (Wilmington, DE).

13. $^{13}C_6$-labeled 3-phenoxybenzoic acid (3-PBA) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA).

14. Nitrogen gas used by the TurboVap LV evaporator had a minimum purity of 99.999% and was purchased from Airgas Inc. (Hapeville, GA).

15. Nitrogen and zero air used by the MS were supplied by a Peak nitrogen/air generator model NM20AZ (Peak Scientific Instruments Ltd., Inchinnan, Renfrew, Scotland).

16. Reagents were prepared with deionized water that was organically and biologically purified with a model D8981 NANOpure Infinity UF water purification system (Barnstead Thermolyne Corporation, Dubuque, IA).

B. Quality Control Materials

Urine was collected from multiple donors, combined and mixed overnight at 4 °C. After pressure filtering with a 0.2 µm filter capsule to remove bioparticulates, the urine was divided into three pools. The first pool (low pool) was spiked with the native materials to yield an approximate concentration of 3 µg/L. The second pool

(high pool) was spiked with the native materials to yield an approximate concentration of 10 µg/L. The third pool was not spiked. After being screened for possible endogenous analytes to ensure the absence of any measurable SU herbicides, it was used as pooled urine for calibration standards and blanks. Quality control materials were stored at -70ºC.

C. Standard Preparation

Stock native spiking standards (non-isotopically labeled) were made by weighing out approximately 1 mg of each solid SU herbicide and dissolving this in acetonitrile. Stocks of all 17 SU herbicides were combined and dilutions were carried out to make eight native spiking standards. From these native spiking standards, eight calibration standards, ranging from 0.05 µg/L to 50 µg/L, were prepared in "blank" urine and analyzed with each analytic run. Their response ratios (area of the analyte quantification ion/area of the ISTD ion) were plotted, and a best fit plot was obtained using a regression analysis. The resulting equation was used to calculate unknown concentrations. The lowest standard concentration was at or below the method limit of detection (LOD) to ensure linearity and accuracy at the low concentration end.

E. Equipment

(1) Water bath (Equate, Curtin Matheson Scientific)
(2) Solid phase extraction vacuum manifold (Supelco, Inc., Bellefonte, Pennsylvania)
(3) Microbalance (Sartorial Ultra micro, Westbury, NY)
(4) Rotator - Glass-Col, RD-230
(5) Centrifuge (IEC Centra-7, International Equipment Co.)
(6) Gilson 215 liquid handler (Gilson, Middleton, WI.)
(7) Turbovap LV Evaporator (Zymark, Hopkinton, Mass.)
(8) HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA), with a chilled (10 °C) auto sampler.
(9) Sciex API4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA,
(10) Autosampler (CTC A200s, Carrboro, N.C.)

F. Other Materials

(1) Round bottom 50 mL screw capped tubes (Kim ax, Scientific Services, CDC).
(2) Conical bottom 15 mL screw capped tubes (Pyrex or Kim ax, Scientific Services, CDC).
(3) Graduated, conical bottom 15 mL tubes (Pyrex or Kim ax, Scientific Services, CDC).
(4) Phenolic screw caps with Teflon seals for both sizes of tubes (Corning, Scientific Services, CDC).
(5) EDP2 Pipettes (Rainin Instrument Co., Woburn, MA).
(6) Pipetman (Gilson Co.).
(7) Vortex Genie (Scientific Industries Inc., Springfield, MA).
(8) Synergi Polar–RP–80A column, 4 µm, 100mm × 4.6mm,(Phenomenex, Torrance, CA)

(9) Micro autosampler vials with aluminum seals (Caltech, Milwaukee, WI)

G. Instrumentation

The high performance liquid chromatography (HPLC)–MS/MS analysis was performed on an HP 1100 liquid chromatograph with a chilled (10 °C) auto sampler, interfaced to a Sciex API4000 triple quadrupole mass spectrometer.

H. Method Validation

Validation results including LOD, regression coefficients, extraction efficiencies, and total (absolute) recoveries of QC materials are summarized in Table 1.

Each validation parameter is explained in more detail in the following paragraphs.

(1) Limits of Detection

A method LOD, based on the precision of measured values (n=10), was calculated for each analyte as $3s_0$, where s_0 is the estimated standard deviation of measured concentration values as the concentration approaches zero[1] With this technique, s_0 is an extrapolated value, and equivalent to the y-intercept of a regression line from the plot of the standard deviations of the measured concentrations versus their nominal concentration values. The three lowest standards (0.05, 0.1 and 0 µg/L) were used for this calculation. Te ability to see each analyte at its calculated LOD by extracting urine samples that were spiked at those concentration levels and injecting them on the instrument was verified

The LOD for all analytes ranged from 0.05 µg/L to 0.10 µg/L. Calibration ranges for all analytes were from LOD to 50 µg/L.

(2) Extraction Efficiency and Total Recoveries

The extraction efficiency of the SPE cartridges, using quality control material, was determined at 2.5 µg/L and 25 µg/L for each analyte by spiking ten "blank" urine samples with the appropriate concentration and extracting according to the method. Ten additional "blank" urine samples (unspiked) were extracted concurrently. Before the evaporation/concentration steps, all of the extracts were spiked with ISTD. Five of the ten samples that were not spiked before preparation were then spiked with 2.5 µg/L and the other five with 25 µg/L, to serve as control samples representative of 100% recovery. After evaporating and reconstituting in solvent, the samples were analyzed. The extraction efficiency of the SPE cartridges was calculated by comparing the response ratios of the urine samples spiked before extraction to the response ratios of the urine samples spiked after the extraction.

The total (absolute) recovery, which included all of the steps of sample preparation, was determined in a similar way except that the spikes representing 100% recovery and the ISTD were added to the injection vial just before injection.

Extraction efficiencies of the SPE cartridges ranged from 79% to 97%. Total recoveries using the method, which included all losses during sample preparation, ranged from 53% to 82%. Analyte concentration did not affect extraction efficiencies or total recoveries. The difference between the total recovery and the extraction efficiency, which reflects the loss of analyte in the evaporation and reconstitution steps, averaged about 15%. However, for two compounds, foramsulfuron and nicosulfuron, the difference was more than 30%.

(3) Precision

The precision of the method was determined by calculating the relative standard deviation (RSD) of repeat measurements (n=50) of quality control materials at two different concentrations (3 µg/L and 10 µg/L). Five samples from each concentration were analyzed on ten different days (50 low values and 50 high values in total). The results were used to determine within-day (WD) and total RSDs for each analyte.

The precisions and accuracies of the QC materials are summarized in Table 2. The average within-day and total RSDs using QC materials were 5% and 8%, respectively, at 10 µg/L. The variation increased at lower concentrations. All analytes had total RSD values <15% except for nicosulfuron. All analytes had accuracies within 15% of the spiked value when using pooled urine, except halosulfuron methyl and nicosulfuron (Table 2).

The precisions and accuracies of discrete urine samples are summarized in Table 3. At the 3 µg/L and 10 µg/L levels, ten of the 17 analytes had total RSDs that exceeded 15%. The decline in precision performance at these levels with discrete urine samples was caused by BM variation and not WM variation. At the 0.1 µg/L level, the total variation exceeded 15% for additional analytes, as would be expected at concentrations near or at the LOD.

(4) Accuracy.

The accuracy was evaluated as the degree of agreement between means of the measured concentrations of samples and their nominal spiked values. The accuracy was determined at two concentrations by comparing mean concentration values of QC samples (n=50 for each concentration) with nominal spiked values (3.0 µg/L and 10 µg/L). Accuracy is calculated as the mean percentage deviation from the spiked value. Mean values deviating not more than 15% from the nominal spiked value were considered acceptable.

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The accuracy of the method for individual compounds was similar regardless of whether the matrix tested was QC material or discrete urine samples.

(5) Matrix Effects

Complex biological matrices, such as urine, include varying endogenous components that affect the accuracy and precision of measured values by suppressing or amplifying the electrospray ionization process. These effects, called “matrix effects”, were evaluated by comparing the accuracy and precision of measured values in urine from 10 individuals spiked at varying concentrations.

Matrix effects on precision and accuracy are attributable to variations in urine matrices, These effects were evaluated at three concentrations (0.1

µg/L ,3 µg/L and 10 µg/L) by spiking ten discrete urine samples with six replicates at each concentration. The spiked urine samples were prepared and analyzed according to the method. Precision parameters including within-matrix (WM), between-matrix (BM) and total RSDs as well as between-matrix accuracy were evaluated to measure the variation in matrix effects and to investigate the method's ability to give quantitative data when analyzing real unknown samples.

(5) Chemical Stability.

The chemical stability of sulfonylurea herbicides was evaluated by storing them in urine and acetonitrile at varying temperatures over several months. The test samples were analyzed periodically to track degradation rates and calculate the half-life for each analyte.

Long-term chemical stability was tested by dissolving the SU herbicides in acetonitrile and urine and storing them at −70 °C, −10 °C, 6 °C, 23 °C, and 37 °C. At several intervals during a ten month period, the mixtures in acetonitrile were removed from storage and allowed to come to room temperature. Aliquots (n=3) from the mixtures stored at each temperature were spiked with labeled 3-PBA to correct for variations in MS/MS response, briefly vortex-mixed, and analyzed on the MS. Response ratios obtained from the −70 °C aliquots were averaged and used as a reference value representing an assumed zero degradation. This assumption was valid if a negligible degradation occurred at −10 °C. At each time point, all other response ratio values were normalized to the −70 °C reference value for that time point. Plots of normalized response ratio values versus days were used to estimate the number of days for 10% and 50% (half-life) degradation of each SU herbicide. Stability tests for SU herbicides stored in urine were performed in a similar way, but all of the samples were spiked the same day and stored in individual screw-capped test tubes so that only the samples tested at each time point would have to be thawed, thus protecting the remaining samples from freeze/thaw cycles. The urine samples also required extraction according to the method before instrument analysis.

The days for 50% degradation (half-lives) at 23 °C and 37 °C and for 10% degradation at −10 °C, 6 °C, and 23 °C, in acetonitrile and urine, were estimated.

(6) Effect of Stability on Measured Values.

Because sulfonylurea herbicides have a tendency to degrade, three experiments to evaluate possible effects on our measured values were conducted:

"Re-inject Repeatability"—same extracts was re-injected and analyzed. Values from re- injections were compared to values from first injection.

To investigate effects on precision and accuracy caused by repetitively injecting an analytic run, ten QC low samples, ten QC high samples, and a complete standard series were prepared. The samples were analyzed the day they were prepared. The samples were then stored at −10 °C overnight. The next day the samples were removed and placed in a chilled (10 °C) autosampler tray. They remained there 24 hours before being analyzed a second time. The injection vials were then stored at −10 °C overnight

before being reconstituted with 15 µL of acetonitrile, briefly vortex-mixed, and injected and analyzed a third and final time. Mean values from the second and third injections were compared to mean values from the first injections. The accuracy of the second and third injections was expressed as percentage deviations from the mean values obtained on the first day.

In the "reinject repeatability" tests, all mean values measured on days after the first day were within 15% of the mean values measured on the first day except for foramsulfuron and nicosulfuron.

"Delayed Injection" —extracts were stored at –10 ºC up to five days before analysis. Values from days two through five were compared to values from day one.

The stability of SU herbicides stored in urine extract for extended periods was evaluated. Six runs consisting of five QC low samples, five QC high samples, and a standard series were prepared concurrently. Runs were combined and thoroughly mixed to insure homogeneity. The combined runs were subsequently aliquoted into six separate runs. One run was analyzed the same day, and the other five runs were stored at −10 °C. Each day following, one run was removed and analyzed. Average measured concentration values from each day were compared with the average measured concentration values from the first day.

In the "delayed injection" tests, all mean values measured on days after the first day were within 15% of the mean values measured on the first day except for foramsulfuron and nicosulfuron.

"Stability-in-Autosampler" —extracts remained in chilled (10 ºC) autosampler tray and were injected over 22 hours. Trends (slopes) in the area counts over the 22 hour period were evaluated as possible degradation of analyte.

The stability of each SU herbicide was evaluated during the time the samples were chilled (10 °C) in the autosampler before injection. Thirty-four high-concentration standards (50 µg/L) were prepared according to the method except that the final reconstituted extracts were combined to make a homogenous mixture and then aliquoted into 34 injection vials. The vials were placed in the chilled auto-sampler tray with two rinse vials between each standard vial and analyzed over a 22-hour period. Regression analysis was performed on the response ratios for each analyte. A slope significantly different from zero representing >5% loss over the time period was considered an indicator of possible degradation.

In the "stability-in-autosampler" test, all analytes had response ratios that varied 5% or less over the 22-hour period except rimsulfuron, which decreased 8%.

(7) Retention time Precision.

The precision of the chromatographic peak retention times was evaluated from 50 injections over 10 days.

All analytes had chromatographic retention times that deviated no more than two seconds from the mean value (n=50).

(8) Adsorption to Glass.

Aliquots from repeated transfers were analyzed to detect any decrease in concentration.

To test the SU herbicides' adsorption to glassware, a 10 µg/mL mixture of the analytes (in acetonitrile) was aliquoted into each type of glassware used in the method and allowed to remain for 15 minutes before being transferred to another glass of the same type. Eight additional transfers, at 15 minute intervals, were made. Initially, and at each transfer, 50 µL aliquots (n=3) were pipetted into injection vials and 50 µL of ISTD was added. For each type of glassware, 27 aliquots were analyzed. The response ratios of each SU herbicide were plotted. Slopes were evaluated using regression analysis. Only statistically significant slopes ($p<0.05$) were considered. Glass adsorption was considered negligible if the total loss after ten transfers was less than 5%.

The glass adsorption test showed one significant negative correlation between response and number of transfers (mesosulfuron methyl in a stock solution container). The total loss after ten transfers was 7%.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

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A. Mass Spectrometer

The API1000 mass spectrometer is calibrated and tuned according to the instructions in the operator's manual. The "OPT" ICL program can be modified and executed to determine the optimum for each parameter. After the instrument is calibrated with unit resolution and maximum sensitivity, the instrument is prepared for analysis of the pesticide metabolites as described in the Procedure Operating Instructions.

B. Calibration Curve

(1) A eight-point calibration curve is constructed by performing a linear reqression analysis of relative response factor (i.e., area native/area label) versus standard concentration. A minimum of five repeat determinations are performed for each point on the standard curve.

(2) The lowest point on the calibration curve is at or below the measurable detection limits and the highest point is above the expected range of results.

(3) The slope and intercept of this curve is determined by linear least squares fit using SAS software.

4) R-squared values for the curve must be greater than 0.90. Linearity of standard curves should extend over the entire standard range. Intercepts, calculated from the least squares fit of the data, should not

be significantly different from 0; if it is, the source of this bias should be identified.

(5) The standard curve should be recalculated periodically to incorporate the newest data points. Whenever a new combined labelled isotope solution is prepared, the standard curve must be re-established.

C. Calibration Verification (CV)

(1) Calibration verification materials are analyzed, using the same procedure used with the unknown samples, after any substantive change.

(2) Calibration verification should be performed a minimum of once every 6 months while the method is in use.

(3) Two CV materials (described in standard preparation section; one standard representing the high detection end of the method; one standard representing the low detection end of the method; are analyzed per calibration verification runs. The slope, intercept, and linearity of a regression analysis of the CV materials should not differ significantly from that of the calibration curve.

(4) If there is a significant difference, analyses using this method should be halted until corrective actions are taken and CV materials are consistent with the calibration curve.

(5) All calibration verification runs and results shall be appropriately documented.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Sample Preparation

Urine samples were frozen within 1 hour of collection and stored at -20 °C before analysis. For each analytical run, calibration samples, two fortified urine samples (one high and one low dose), two "blank" urine samples, and one solvent blank were prepared, extracted, and analyzed in parallel with the unknown samples.

B. Sample Cleanup

Urine(2 mL) was pipetted into 20 mL screw -capped test tubes and spiked with 25 µL of internal standard(ISTD), resulting in a concentration of about 6 µg/ L. After adding 1.5 mL of acetate buffer (pH 5), the sample tubes were briefly vortex-mixed before being extracted with Oasis HLB 3 cc solid phase extraction (SPE) cartridges (Waters Corporation, Milford, MA). The SPE cartridges were first conditioned with 1 mL of methanol followed by 1 mL water. After the

samples were loaded onto the cartridges, they were washed with 1 mL of 5% methanol in water. Samples were eluted with 2 mL of 100% methanol into 20 mL conical tubes. Sample extracts were concentrated to dryness at 40 °C using 10 psi of nitrogen for 30 minutes. After concentration, residual SU herbicides were rinsed from the walls of the test tubes by adding 0.35 mL of 100% methanol to each tube and vortex mixing for 5 seconds. After concentrating for seven additional minutes, samples were reconstituted with 50 µL of acetonitrile, briefly vortex-mixed, and transferred to autoinjection vials for analysis.

C. Instrumentation

The high performance liquid chromatography (HPLC)–MS/MS analysis was performed on an HP 1100 liquid chromatograph with a chilled (10 °C) auto sampler, interfaced to a Sciex API4000 triple quadrupole mass spectrometer.

D. Daily operating protocol

A typical analytic run consists of eight standards (a standard series), two QC samples, two blank samples, and 38 unknown samples. For this method, which had an analysis time including column re-equilibration of approximately 12.5 minutes per sample, the total run time would be approximately 10.5 hours. To evaluate for possible degradation during that period, the response ratios of each analyte over a much longer time of 22 hours. The response ratios for each analyte varied on average by 2.8% over 22 hours was monitored. For a typical analytic run time of 10.5 hours, the average variation would be about 1.4%. By placing the standard series in the middle of the analytic run, the effect on measured values could be reduced to an average of about 0.7% per run. Therefore, under normal operating conditions, (50 samples in an analytic run), the time the samples spend in a chilled (10 °C) autosampler awaiting injection should introduce negligible error.

E. GC and MS Conditions

Ten microliters were injected and analytes were chromatographically separated using a Synergi Polar–RP–80A column, 4 µm, 100 mm × 4.6 mm, which was held constant at 35 °C. The flow rate was 1 mL per minute. Initial mobile phase conditions were 55% A (water with 0.1% acetic acid) and 45% B (acetonitrile with 0.1% acetic acid). The analytes were separated using a gradient elution. From 0 min to 6.5 min, B was increased to 64%. From 6.5 to 6.6 min B was increased to 100% and held until 7.5 min. From 7.5 min to 7.6 min, B was decreased back to the starting conditions for a 3.9-min column equilibration period. Analyte retention times were between 2 min and 6.5 min. The MS was operated in the selected reaction monitoring (SRM) mode using negative turboionspray (TIS) atmospheric pressure ionization (API). The TIS heater temperature was 650 °C and the gas pressures of the collision-activated dissociation (CAD), nebulizer, and heater gases were 7 psi, 18 psi, and 15 psi, respectively. Zero air was used for CAD, nebulizer, and heater gases. Nitrogen was used as curtain gas at 35 psi.

G. Processing of data

(1) Quantification

All analytes were quantified using stable isotopically labeled ethametsulfuron methyl as an ISTD. Stock native spiking standards

(non-isotopically labeled) were made by weighing out approximately 1 mg of each solid SU herbicide and dissolving this in acetonitrile. Stocks of all 17 SU herbicides were combined and dilutions were carried out to make eight native spiking standards. From these native spiking standards, eight calibration standards, ranging from 0.05 µg/L to 50 µg/L, were prepared in blank urine and analyzed with each analytic run. Their response ratios (area of the analyte quantification ion/area of the ISTD ion) were plotted, and a best fit plot was obtained using a regression analysis. The resulting equation was used to calculate unknown concentrations. The lowest standard concentration was at or below the method limit of detection (LOD) to ensure linearity and accuracy at the low concentration end.

9. REPORTABLE RANGE OF RESULTS

The linear range of the standard calibration curves determines the highest and lowest analytical values of an analyte that are reportable. The calibration verification of the method encompasses this reportable range. However, urine samples with analytical data values exceeding the highest reportable limit may be diluted and reanalyzed so that the result will be in the reportable range.

A Linearity Limits: Analytical standards were linear for all analytes through the range of concentrations evaluated. Urine samples, whose concentrations exceed these ranges, must be resampled and reanalyzed using a smaller aliquot.

B. Analytical Sensitivity: The detection limits for all analytes was calculated as $3S_0$, where S_0 is the standard deviation at zero concentration, and is determined by linear regression analysis of the absolute standard deviation vs concentration.

C. Accuracy: The accuracy of this method was determined by enriching urine samples with known concentrations of the pesticide residues and comparing the calculated and expected concentrations. The accuracy was consistent across the entire linear range. The accuracy can be expressed as the slope of a linear regression analysis of the expected value versus the calculated value. A slope of 1.0 indicates the results are identical. Another way of expressing a method's accuracy is as a percentage of the expected value.

10. QUALITY CONTROL (QC) PROCEDURES

A. Quality Control Material. The control materials used for each unknown run were urine pools enriched with known amounts of pesticide residues.

B. Collection of Urine for QC Pools. Two quality control pools were prepared and are used in each run of unknown samples. The urine for each pool was

collected from volunteers and was screened to ensure that the endogenous levels of pesticide residues were low or nondetectable. The urine samples were combined and homogenized to form a base pool.

C. Urine Enrichment. The first pool (low pool) was spiked with the native materials to yield an approximate concentration of 3 µg/L. The second pool (high pool) was spiked with the native materials to yield an approximate concentration of 10 µg/L. The third pool was not spiked. After being screened for possible endogenous analytes to ensure the absence of any measurable SU herbicides, it was used as pooled urine for calibration standards and blanks. Quality control materials were stored at -70ºC.

D. Filtration and Dispensing. Each pool was clean filtered to 0.2 µ. The urine was dispensed in 12-mL aliquots into 25-mL sterile screw-capped vials. The vials labeled appropriately and the QC materials were then frozen at -20 °C until needed.

E. Characterization of QC Materials. The QC pools (including the unspiked pool) were characterized by 20 consecutive runs of each QC material. Using the data from these runs, the mean and upper and lower 99^{th} and 95^{th} confidence intervals were established. The confidence intervals were determined and adjusted according to the number of each QC material analyzed in each run.

F. Use of Quality Control Samples. During each analytical run, two blank urines and two QC materials are analyzed. The QC materials can be any combination of the high and low pools.

G. Final Evaluation of Quality Control Results. An analytical run is considered "out-of-control" if the mean QC value or QC range values (for multiple QCs) are outside the 99% confidence intervals. If two consecutive mean QC values or QC range values are outside the 95% confidence intervals, the second of those runs is considered "out-of-control". Any data generated from a run that is not in control are not reported. If more than 8 consecutive QCs are on the same side of the mean of the characterized QC material, all operations will be suspended until it is determined whether a bias is present in the method. This is a preventative measure only; the run is not considered "out-of-control".

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If the calibration or QC systems fail, all operations are suspended until the source or cause of failure is identified and corrected. If the source of failure is easily identifiable, for instance, failure of the mass spectrometer or a pipetting error, the problem is immediately corrected. Otherwise, fresh reagents are prepared and the mass spectrometer system is cleaned. Before beginning another analytical run, several QC materials (in the case of QC failure) or calibration verification

samples (in the case of calibration failure) are reanalyzed. After calibration or quality control has been reestablished, analytical runs may be resumed.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

There are two primary reasons for expanding our validation parameters beyond those of the traditional approach. First, methods developed in this laboratory are typically used in epidemiologic exposure studies which benefit from high levels of precision and accuracy. Ensuring such levels of quantitative performance while measuring SU herbicides, a class of compounds known to be chemically and thermally unstable, warranted more validation tests than most published methods typically include. Second, this method was developed to measure SU herbicides in urine, a complex biologic matrix, which contains endogenous components that can adversely affect the accuracy and precision of measured values. These endogenous components vary from sample to sample and their adverse effects are often only apparent when measuring discrete urine samples. The traditional approach of repetitively measuring pooled matrix material to evaluate the performance of a method masks these adverse matrix effects by averaging the effect of each donor's urine. We, therefore, evaluated the precision and accuracy of the method with pooled material to show the performance of the methodology without considering variable matrix effects (such as precision of pipetting, extraction efficiency, recovery, instrument variation, and so on) and with discrete urine to show the level of performance that would be expected if measuring unknown samples, which would include the additional source of variation caused by varying endogenous materials unique to each person's urine sample.

One of the challenges with accurately measuring analytes in urine is that the matrices of unknown samples are never exactly the same. Urine varies within a person and from person to person, and in fact, every individual produces urine that has a unique combination of varying endogenous components.

Unfortunately, these components can cause variations in critical areas of the measurement process, including the extraction efficiency, total recovery, and instrument response. Variations in one or more of these areas, if not corrected for, can result in bias or errors in the final calculated value. The magnitudes of these matrix-induced errors are directly related to the level of chemical similarity, or behavior, between the ISTD and the analyte being measured

13. REFERENCE RANGES (NORMAL VALUES)

Reference values were determined in the Priority Toxicant Reference Range Study. This study was performed to provide reference values in the human population to determine length or severity of an exposure incidence.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

These measurements require significant time for completion. It is unlikely that any result would be a "critical call", which would only be observed in poisonings.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Urine samples may be stored overnight in refrigeration to expedite thawing prior to aliquoting the sample. The urine extracts are stored in autosampler vials in a -20 °C freezer after analysis.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

If testing cannot be performed, the specimens are stored at -70°C.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Once the validity of the data has been established by the QC/QA system outlined above and has been verified by a DLS statistician, one hardcopy and one electronic copy of the data will be generated. This data, a cover letter, and a table of method specifications and reference range values will be routed through the appropriate channels for approval (i.e. supervisor, branch chief, division director). Once approved at the division level, they will be sent to the contact person who requested the analyses

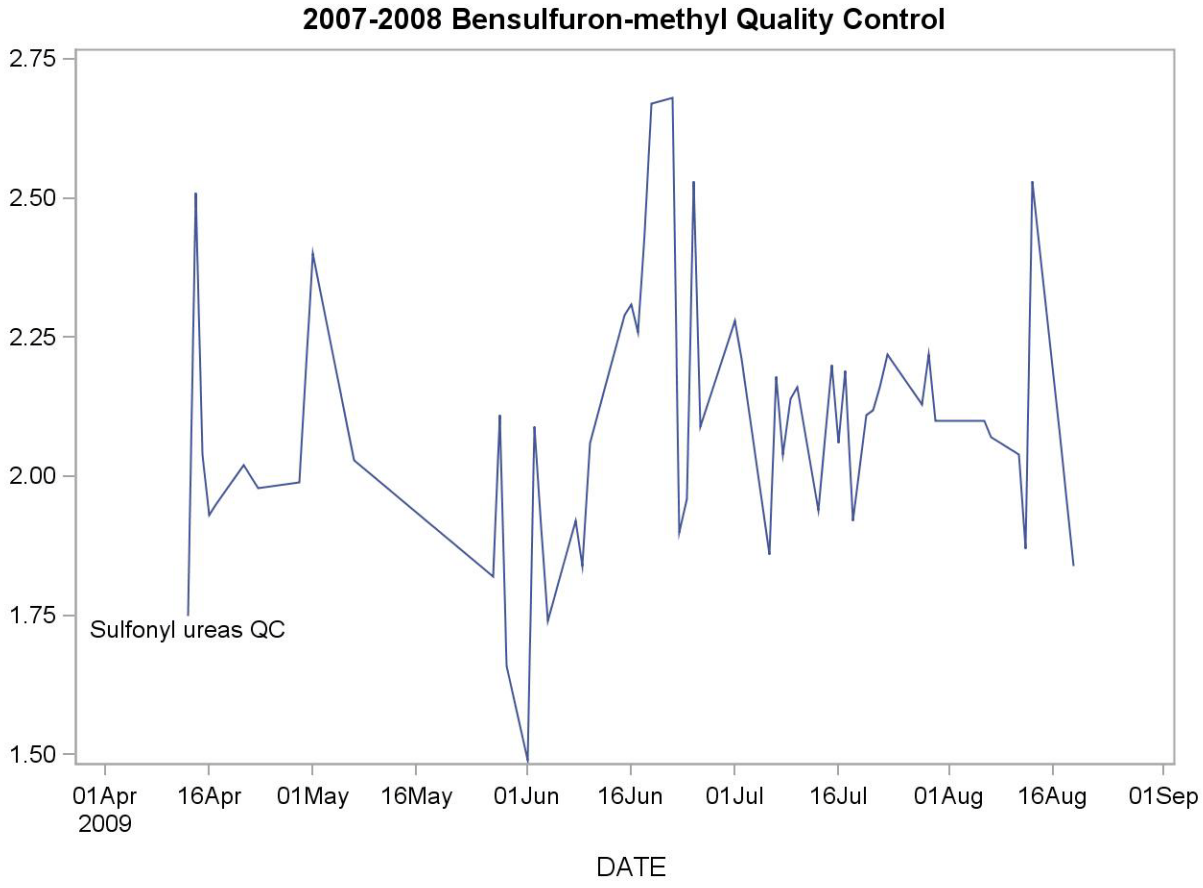
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Standard record keeping systems (i.e. notebooks, sample logs, data files, creatinine logs, demographic logs) should be employed to keep track of all specimens. Specimens will only be transferred or referred to CLIA certified laboratories.

19. SUMMARY STATISTICS AND QC GRAPHS

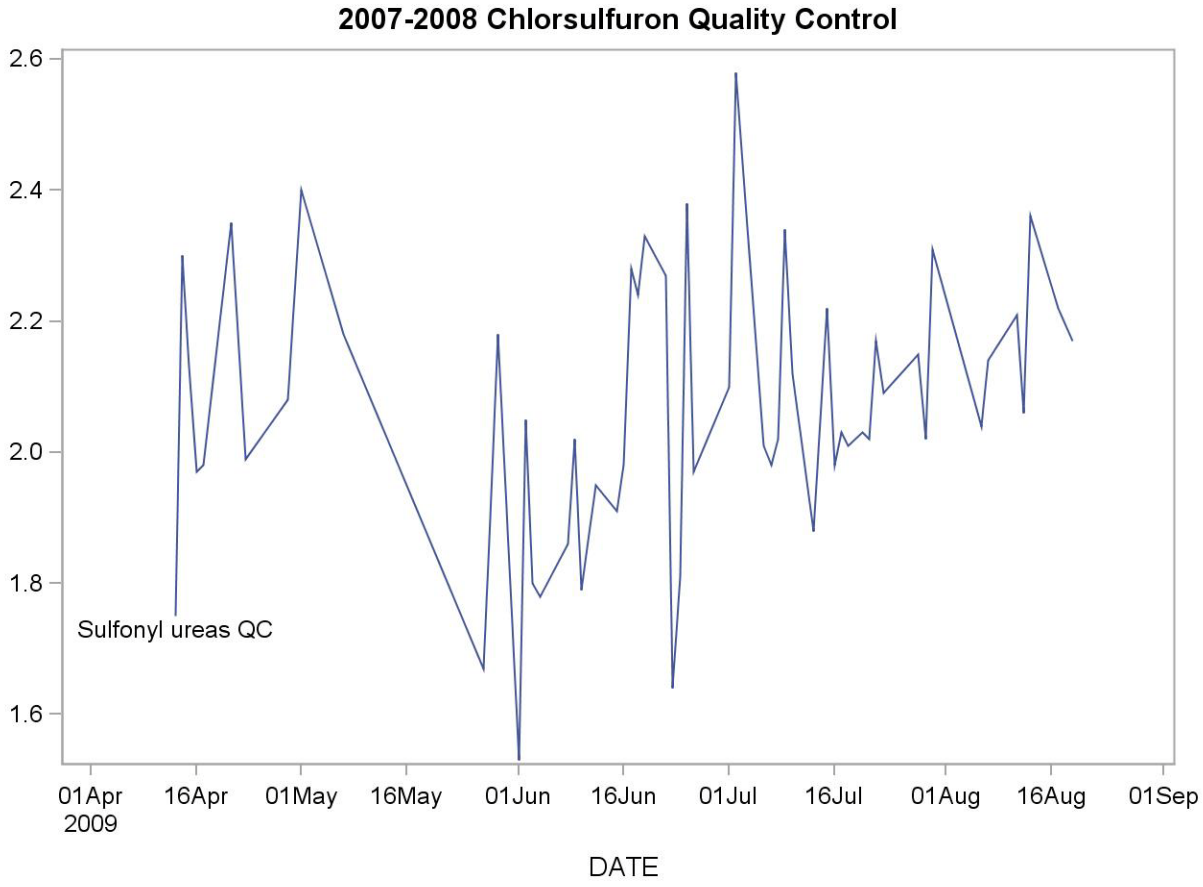
Summary Statistics for Bensulfuron-methyl

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	6.1310	0.7793	12.7
Sulfonyl ureas QC	71	13APR09	19AUG09	0.5577	0.0588	10.5
Sulfonyl ureas QC	71	13APR09	19AUG09	2.0606	0.2333	11.3



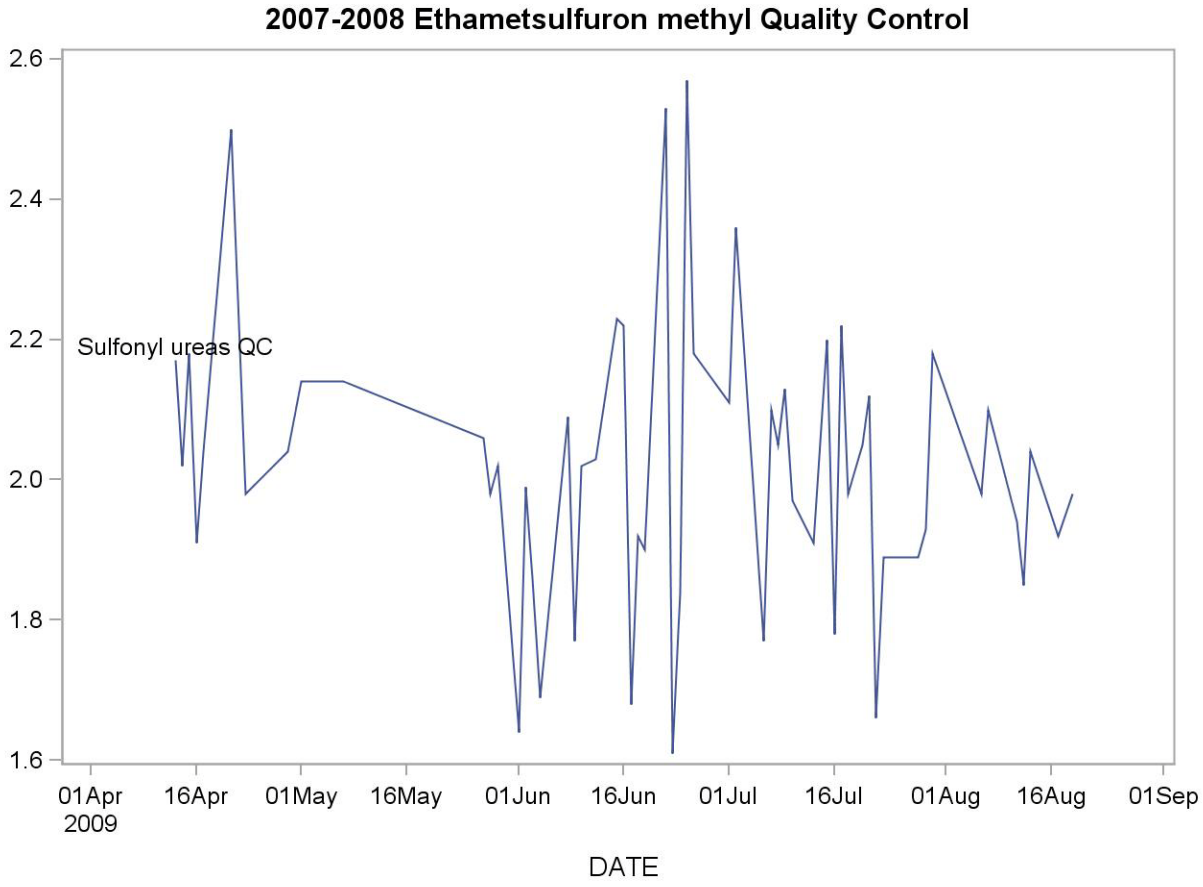
Summary Statistics for Chlorsulfuron

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	5.8183	0.7491	12.9
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5416	0.0533	9.8
Sulfonyl ureas QC	71	13APR09	19AUG09	2.0320	0.2090	10.3



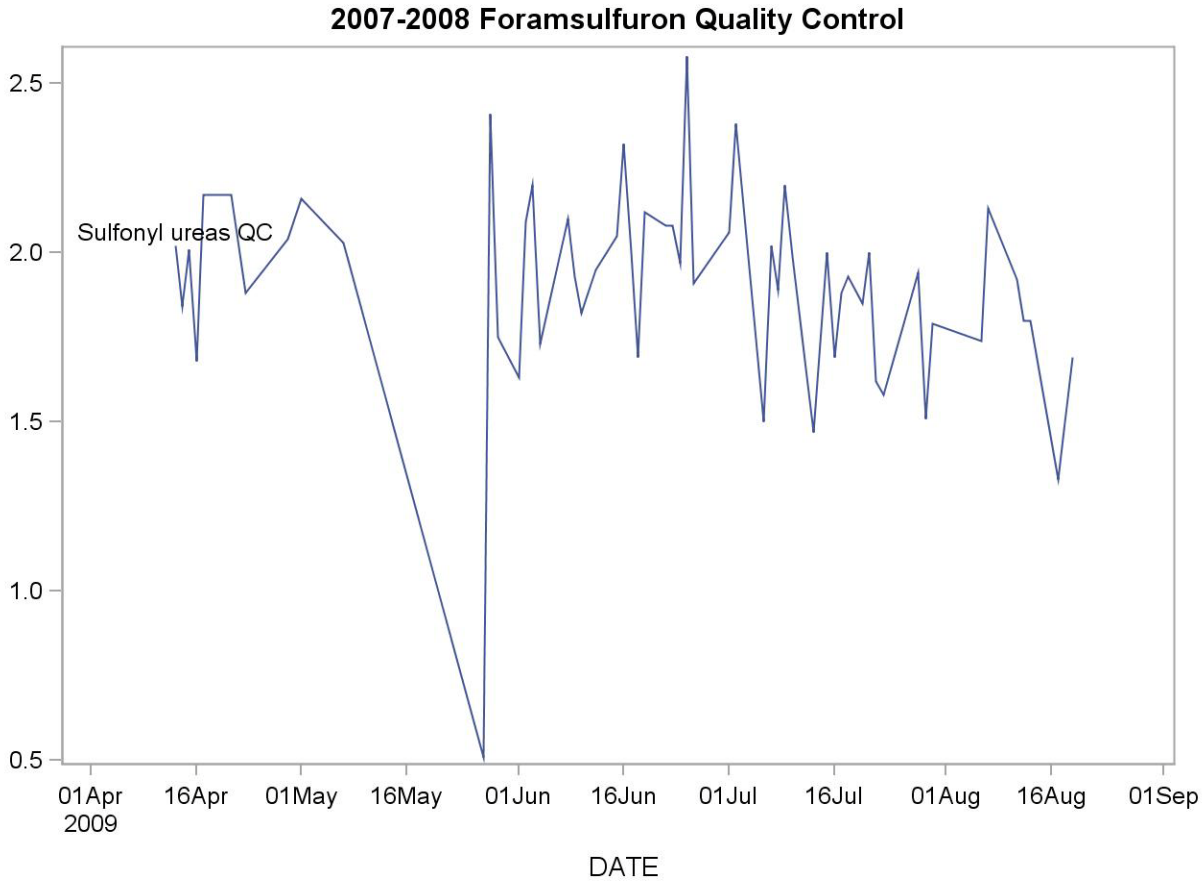
Summary Statistics for Ethametsulfuron methyl

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	68	13APR09	19AUG09	5.8185	0.4780	8.2
Sulfonyl ureas QC	71	13APR09	19AUG09	0.5488	0.0439	8.0
Sulfonyl ureas QC	72	13APR09	19AUG09	2.0013	0.1935	9.7



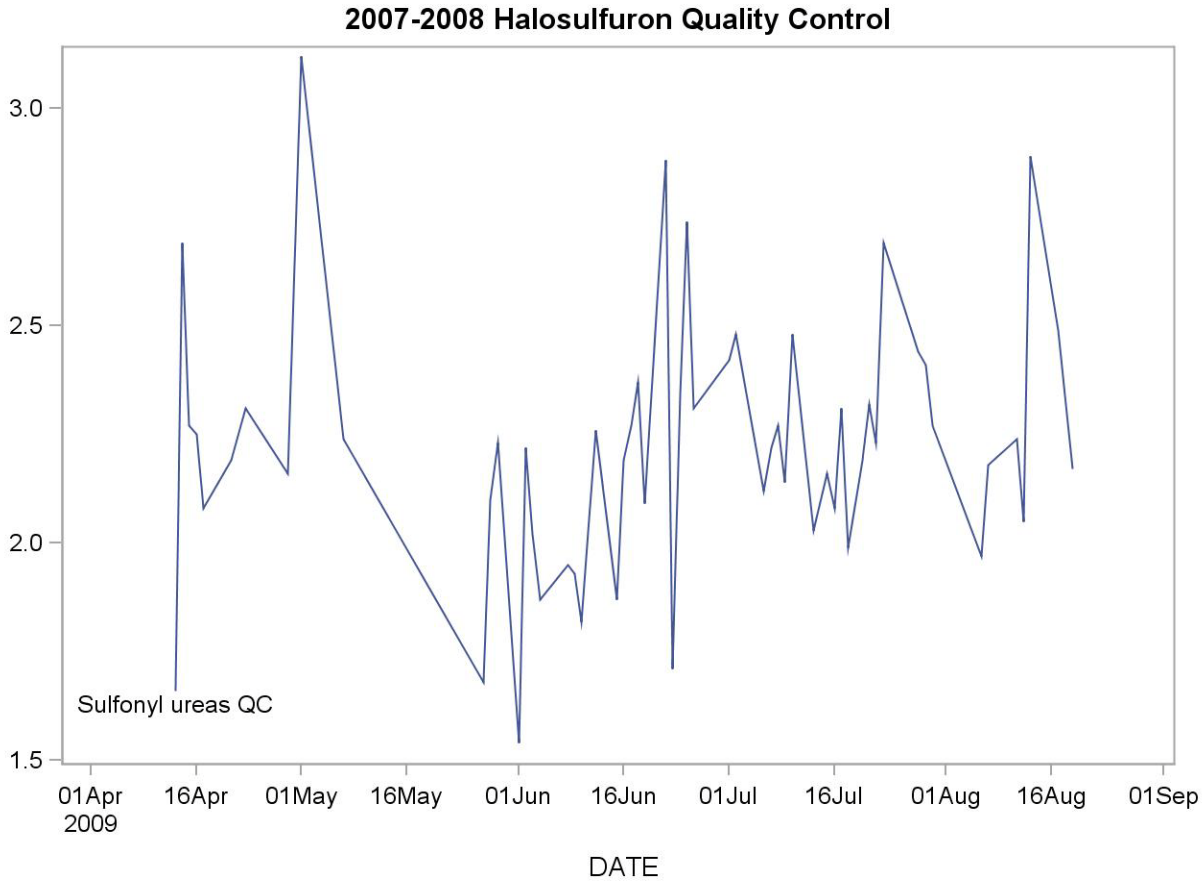
Summary Statistics for Foramsulfuron

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	71	13APR09	19AUG09	5.7832	0.9621	16.6
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5352	0.0810	15.1
Sulfonyl ureas QC	68	13APR09	19AUG09	1.9044	0.2324	12.2



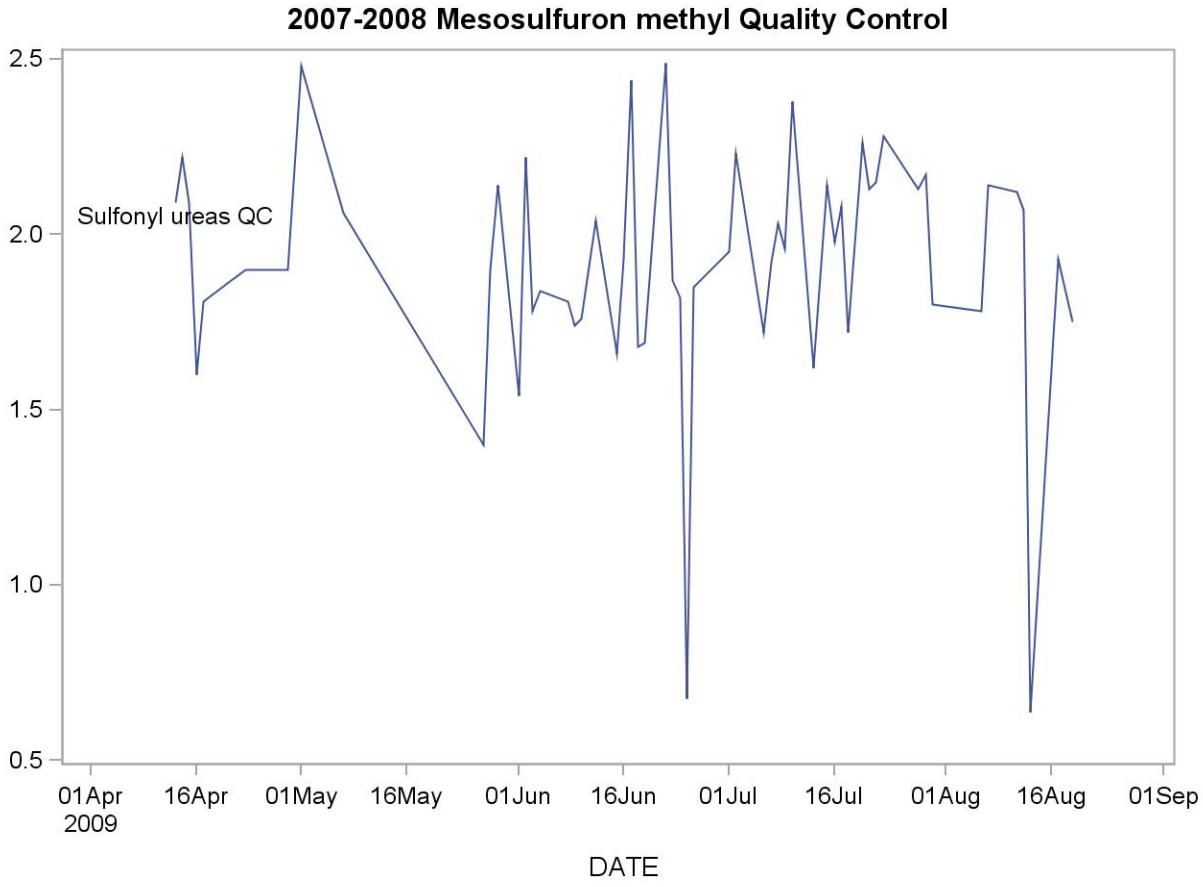
Summary Statistics for Halosulfuron

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	71	13APR09	19AUG09	6.1446	1.0274	16.7
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5950	0.0826	13.9
Sulfonyl ureas QC	71	13APR09	19AUG09	2.1811	0.3036	13.9



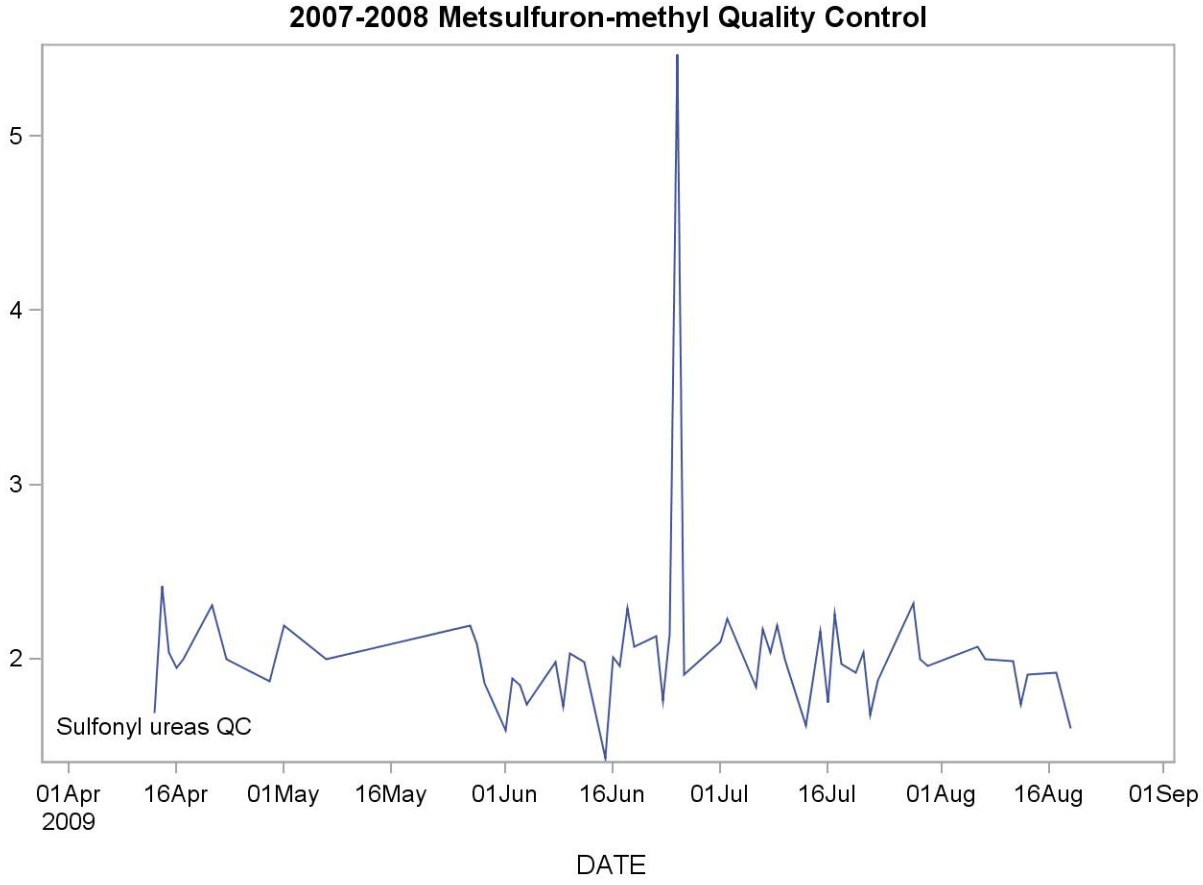
Summary Statistics for Mesosulfuron methyl

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	5.7917	0.7178	12.4
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5487	0.0640	11.7
Sulfonyl ureas QC	69	13APR09	19AUG09	1.9355	0.2397	12.4



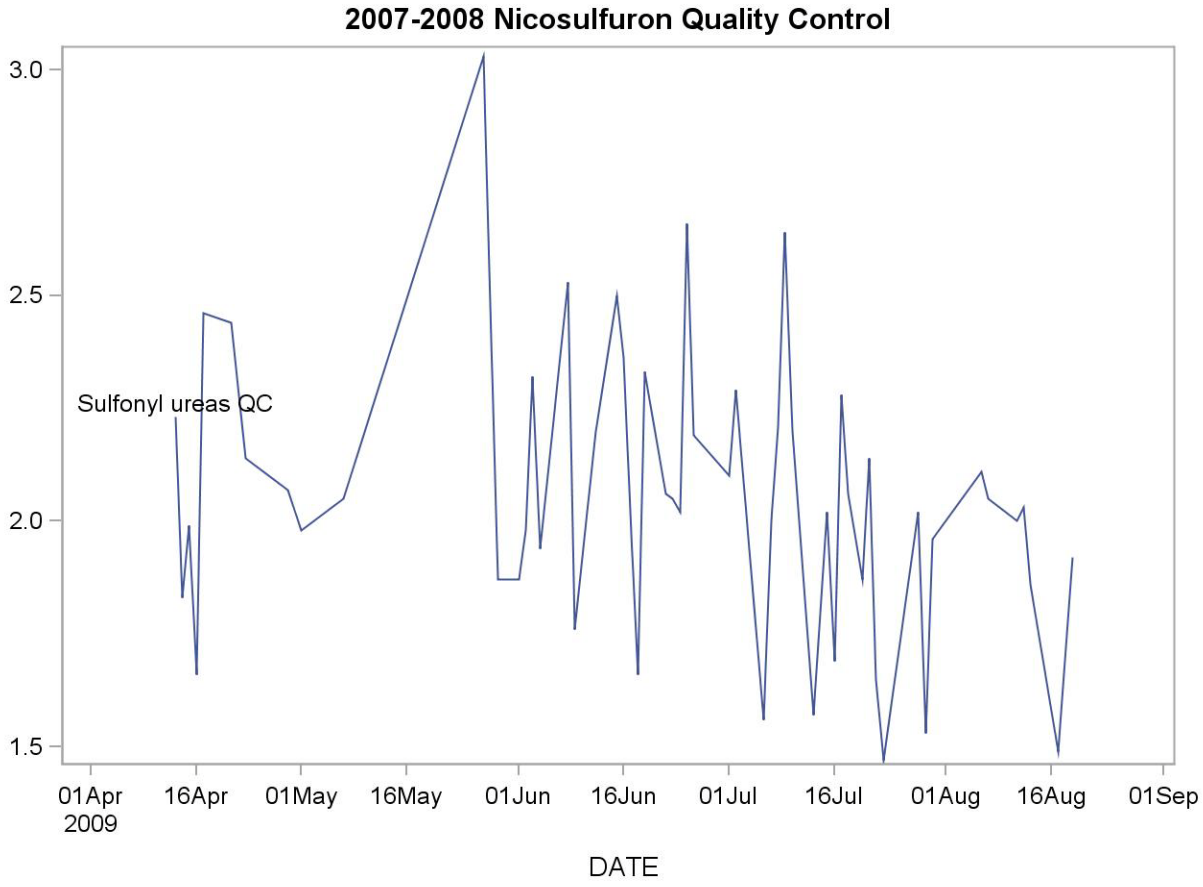
Summary Statistics for Metsulfuron-methyl

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	5.6372	0.7294	12.9
Sulfonyl ureas QC	71	13APR09	19AUG09	0.5254	0.0499	9.5
Sulfonyl ureas QC	70	13APR09	19AUG09	1.9430	0.2022	10.4



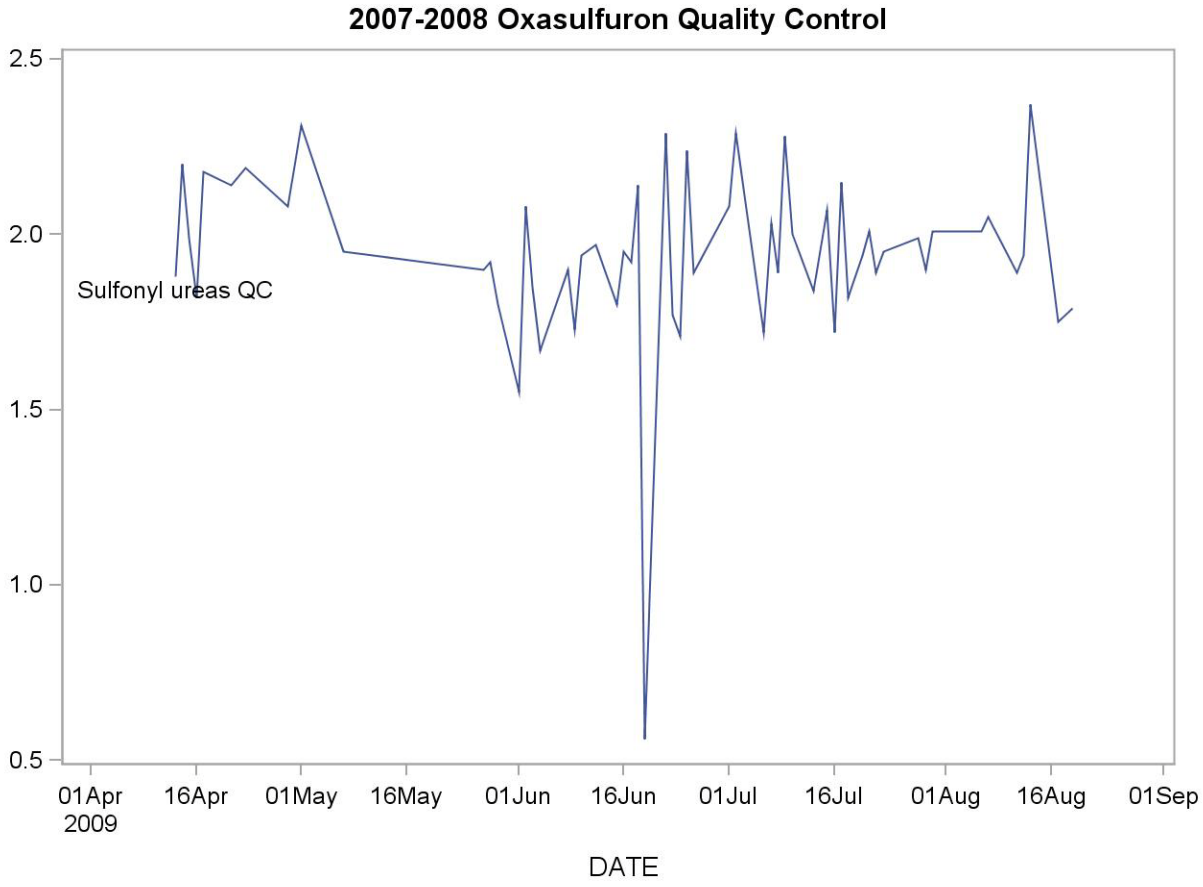
Summary Statistics for Nicosulfuron

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	70	13APR09	19AUG09	5.8761	1.1221	19.1
Sulfonyl ureas QC	71	13APR09	19AUG09	0.5600	0.0993	17.7
Sulfonyl ureas QC	70	13APR09	19AUG09	2.0307	0.2944	14.5



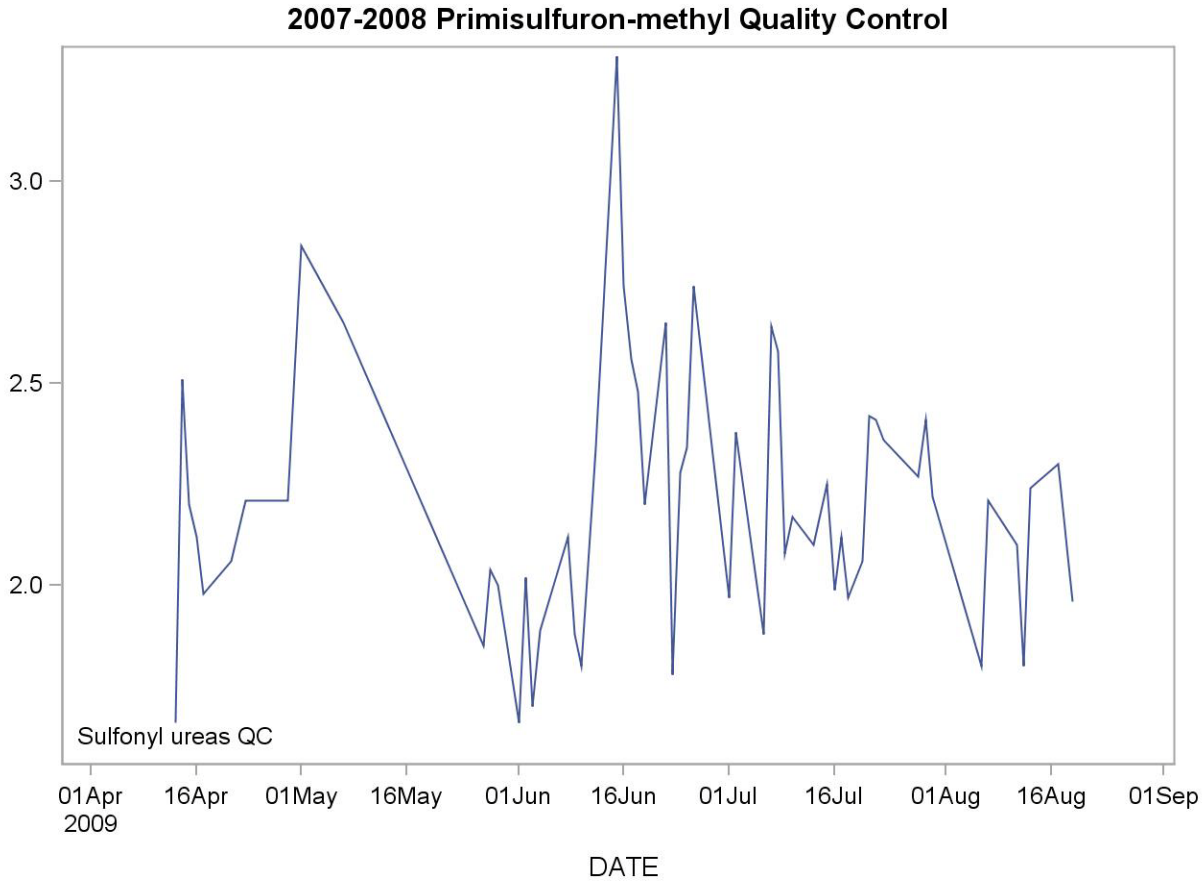
Summary Statistics for Oxasulfuron

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	5.6193	0.5300	9.4
Sulfonyl ureas QC	71	13APR09	19AUG09	0.5324	0.0437	8.2
Sulfonyl ureas QC	71	13APR09	19AUG09	1.9370	0.1799	9.3



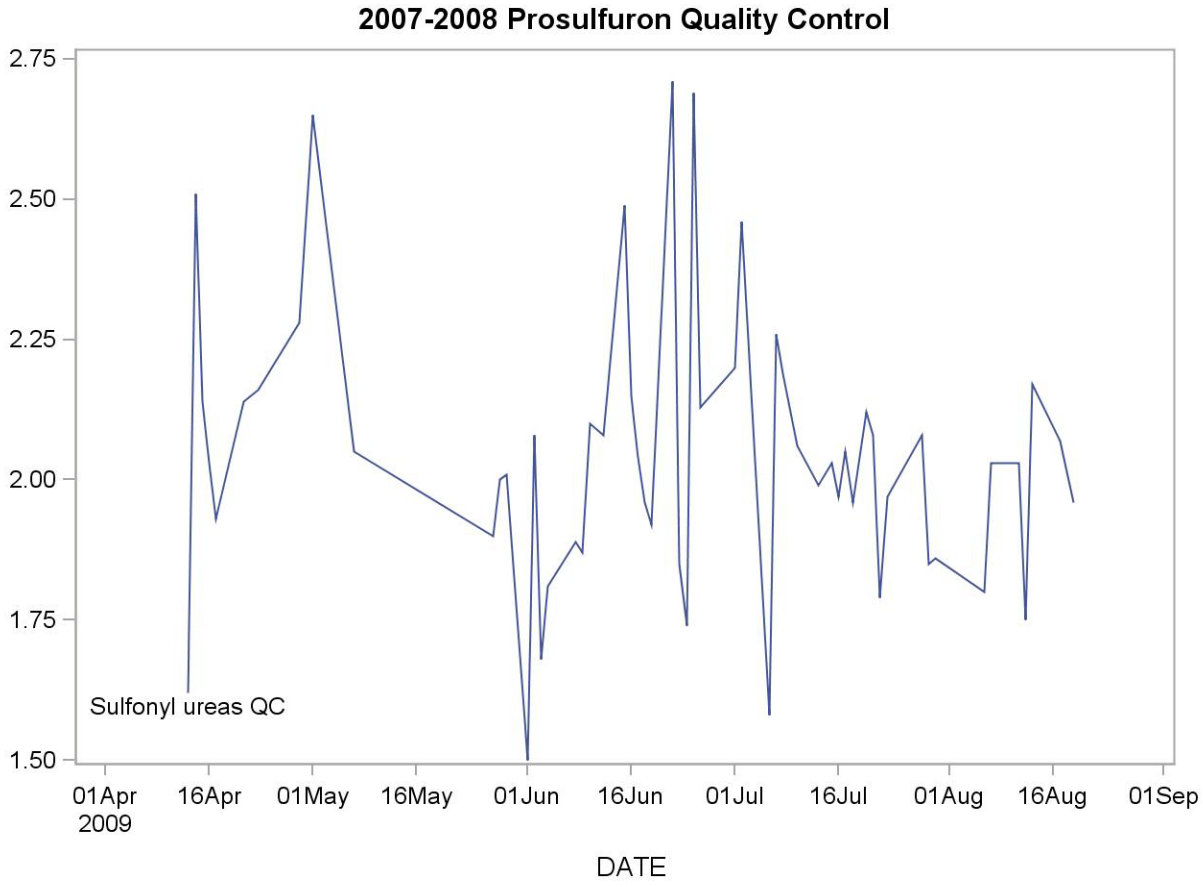
Summary Statistics for Primisulfuron-methyl

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	70	13APR09	19AUG09	6.0603	1.0519	17.4
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5607	0.0807	14.4
Sulfonyl ureas QC	70	13APR09	19AUG09	2.1719	0.3274	15.1



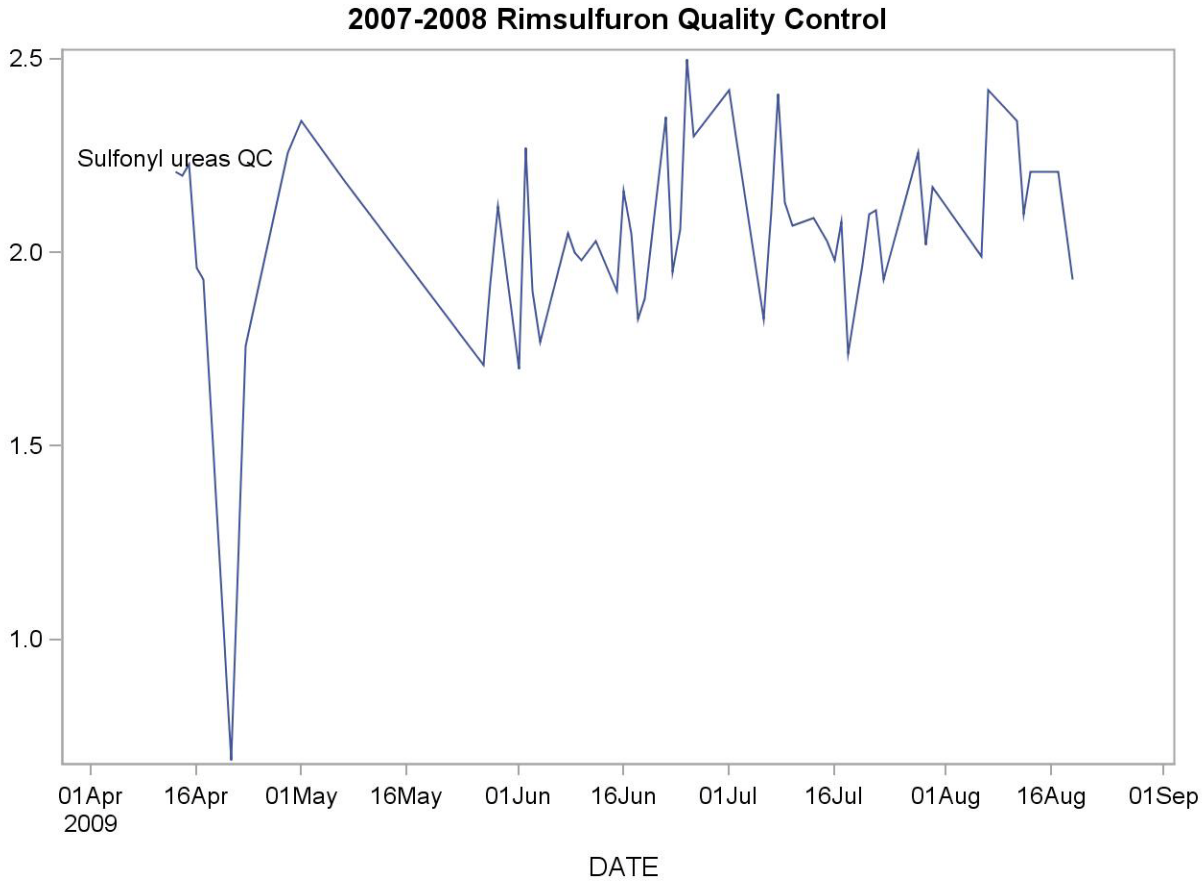
Summary Statistics for Prosulfuron

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	5.8903	0.8132	13.8
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5396	0.0591	10.9
Sulfonyl ureas QC	72	13APR09	19AUG09	2.0251	0.2363	11.7



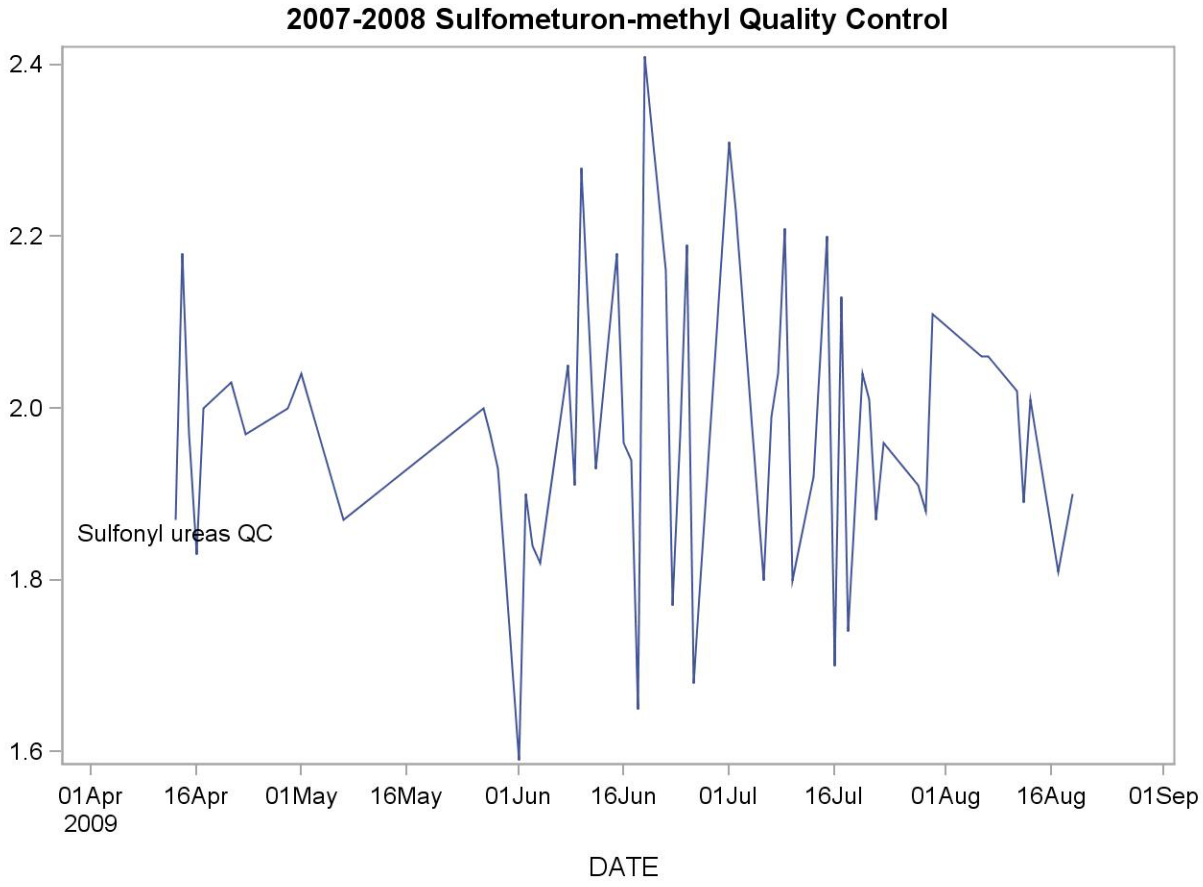
Summary Statistics for Rimsulfuron

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	5.8806	0.6509	11.1
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5643	0.0633	11.2
Sulfonyl ureas QC	71	13APR09	19AUG09	2.0468	0.2023	9.9



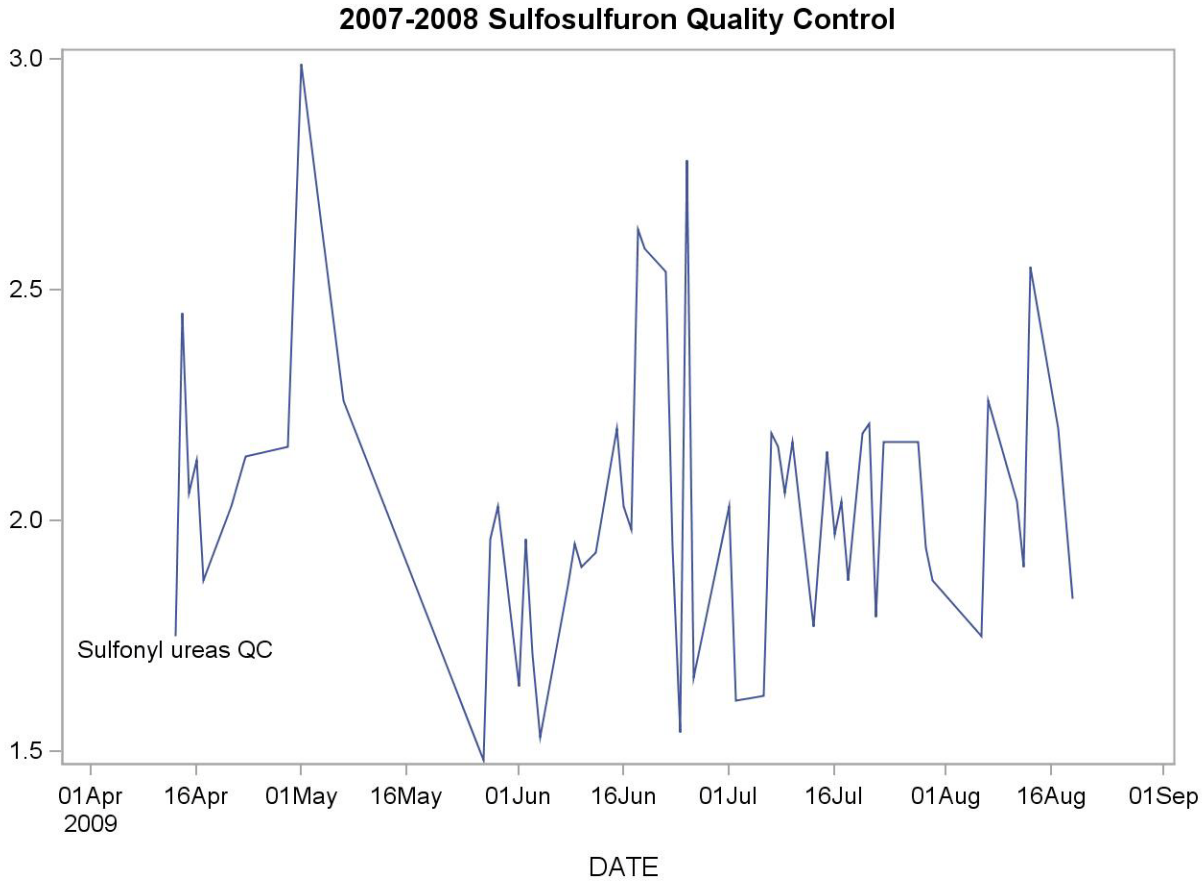
Summary Statistics for Sulfometuron-methyl

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	71	13APR09	19AUG09	5.5007	0.4972	9.0
Sulfonyl ureas QC	71	13APR09	19AUG09	0.5150	0.0558	10.8
Sulfonyl ureas QC	71	13APR09	19AUG09	1.9510	0.1668	8.6



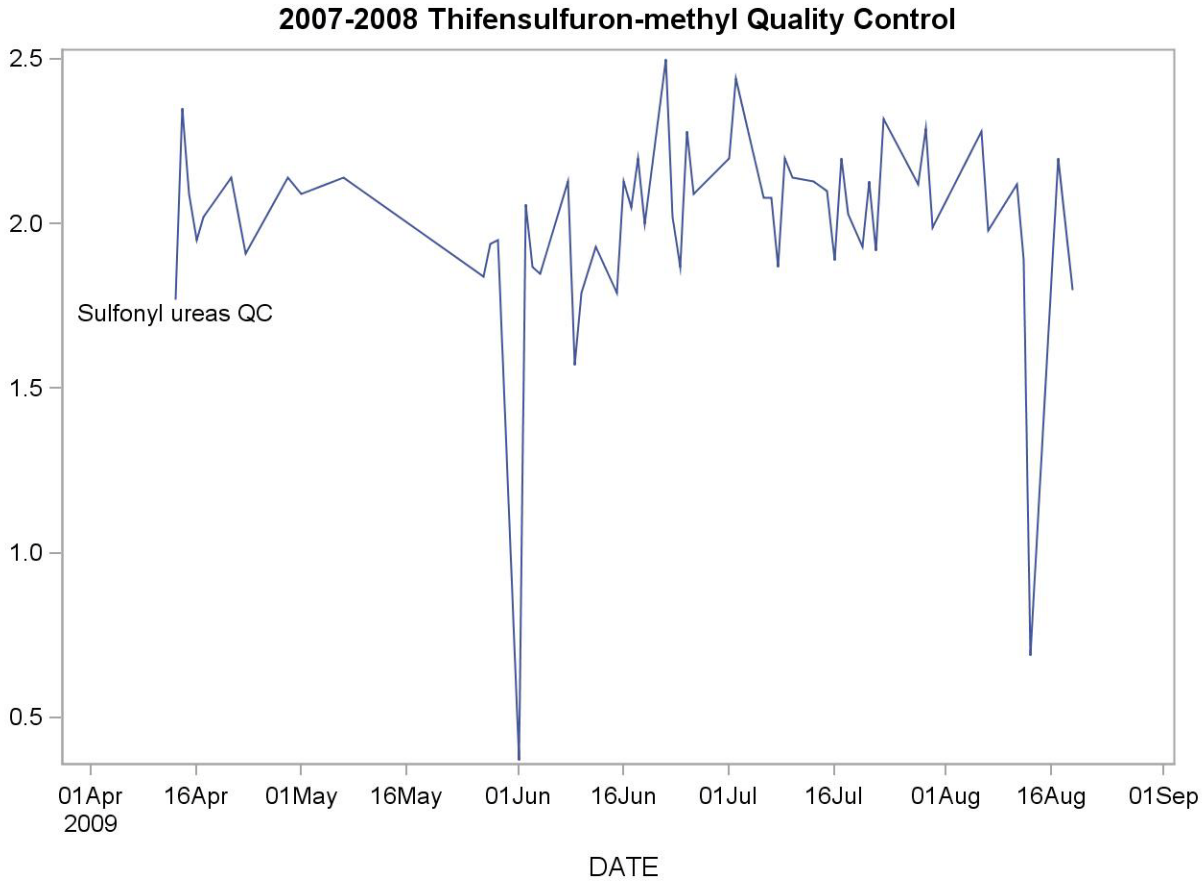
Summary Statistics for Sulfosulfuron

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	6.0218	0.9018	15.0
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5442	0.0606	11.1
Sulfonyl ureas QC	70	13APR09	19AUG09	2.0087	0.2909	14.5



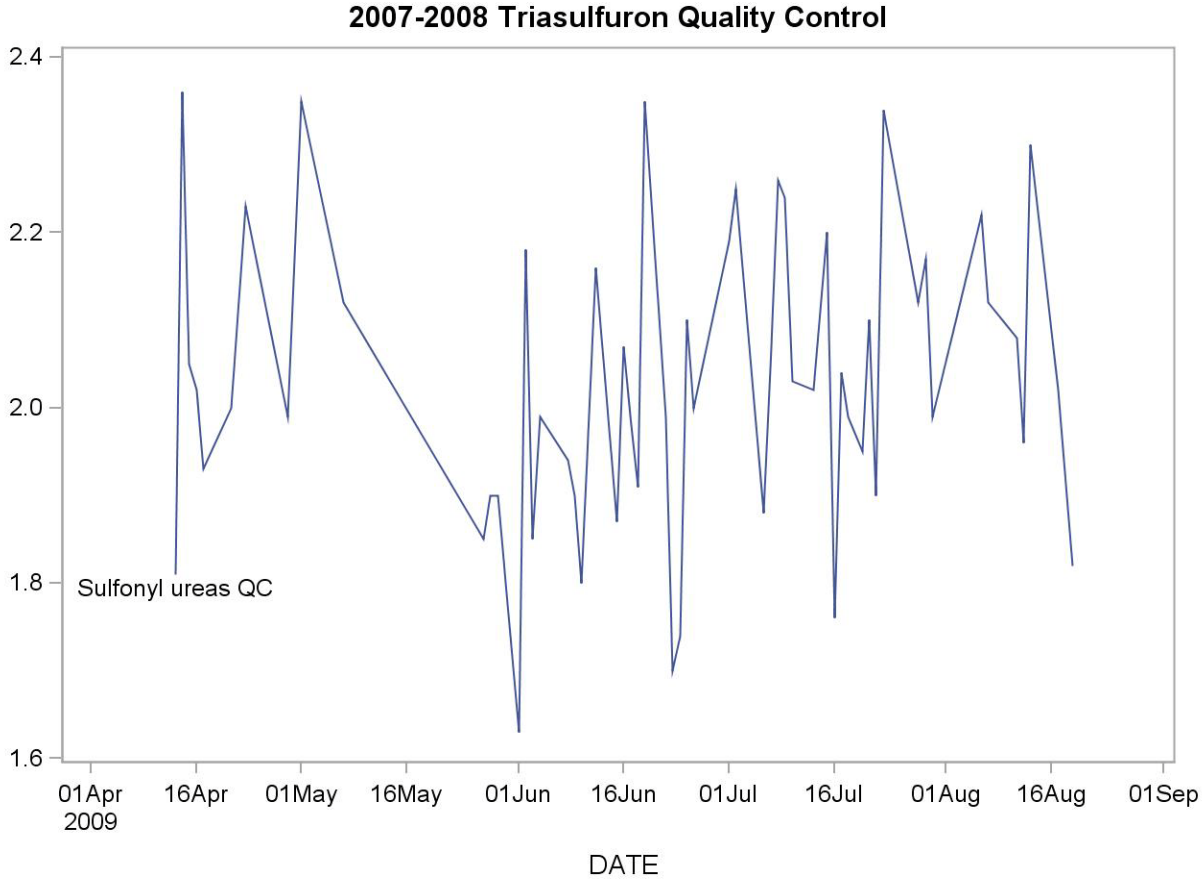
Summary Statistics for Thifensulfuron-methyl

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	5.8500	0.6922	11.8
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5478	0.0511	9.3
Sulfonyl ureas QC	70	13APR09	19AUG09	2.0156	0.1780	8.8



Summary Statistics for Triasulfuron

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	5.8079	0.6075	10.5
Sulfonyl ureas QC	72	13APR09	19AUG09	0.5330	0.0513	9.6
Sulfonyl ureas QC	70	13APR09	19AUG09	2.0019	0.1746	8.7



Summary Statistics for Triflusulfuron-methyl

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
Sulfonyl ureas QC	72	13APR09	19AUG09	5.9036	0.8053	13.6
Sulfonyl ureas QC	70	13APR09	19AUG09	0.5542	0.0545	9.8
Sulfonyl ureas QC	70	13APR09	19AUG09	2.0539	0.2286	11.1

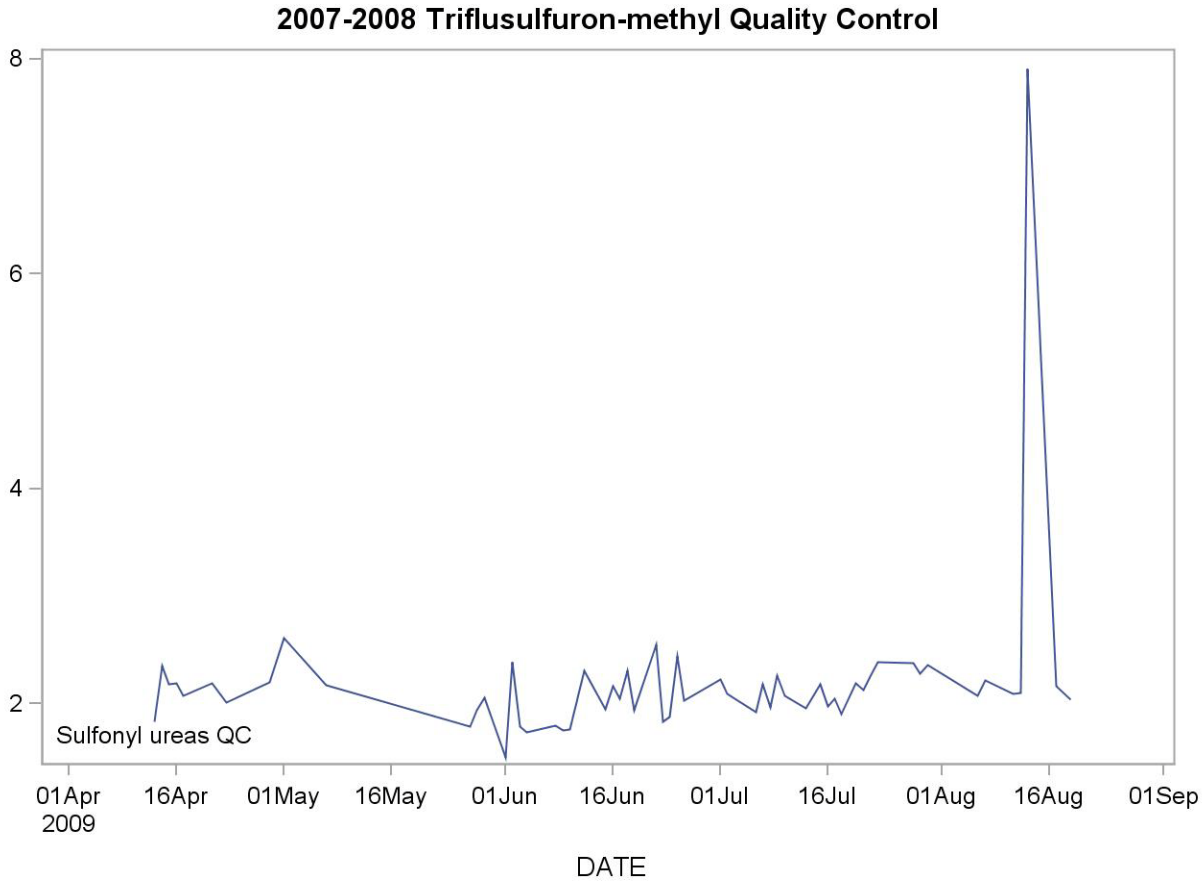


Table 1 Limit of detection, correlation coefficient of regression, extraction efficiency of SPE cartridges and total recovery of pooled urine used for quality control material

Analyte	LOD (µg/L) n=10 %	R^2 (SD) (n=10)	SPE extraction efficiency		Total recovery		
			2.5 µg/L % (stdev)	25 µg/L % (stdev)	2.5 µg/L % (stdev)		25 µg/L (stdev)
Ben	0.05	0.997	(0.003)	85 (6.8)	82 (4.2)	76 (6.2)	72 (3.6)
Chlor	0.05	0.996	(0.003)	90 (6.9)	83 (5.5)	79 (4.3)	72 (5.4)
Etha	0.10	0.998	(0.001)	90 (7.6)	90 (5.0)	70 (6.1)	70 (6.3)
Foram	0.06	0.991	(0.007)	83 (3.8)	86 (7.4)	53 6.9)	53 (3.9)
Halo	0.07	0.991	(0.007)	84 (6.9)	84 (5.0)	80 (5.5)	76 (3.4)
Meso	0.05	0.987	(0.011)	86 (4.8)	90 (8.3)	71 (9.0)	71 (5.6)
Metsu	0.05	0.997	(0.002)	91 (5.3)	88 (7.2)	76 (5.8)	73 (4.7)
Nico	0.10	0.987	(0.010)	89 (3.2)	89 (8.3)	59 (7.2)	60 (6.1)
Oxa	0.06	0.996	(0.003)	90 (5.0)	86 (5.3)	70 (6.0)	67 (5.2)
Primi	0.06	0.995	(0.004)	79 (5.7)	84 (5.6)	72 (6.3)	72 (2.9)
Pros	0.05	0.998	(0.002)	87 (6.2)	86 (5.3)	78 (5.9)	75 (4.3)
Rim	0.06	0.995	(0.003)	90 (5.8)	88 (4.9)	75 (4.4)	72 (5.6)
Sulfom	0.05	0.997	(0.002)	88 (5.8)	88 (3.7)	71 (5.1)	69 (3.7)
Sulfos	0.05	0.996	(0004)	85 (7.0)	82 (5.8)	75 (5.4)	72 (5.9)
Thifen	0.08	0.996	(0.002)	97 (9.7)	87 (5.2)	82 (9.2)	71 (3.7)
Tria	0.07	0.998	(0.001)	87 (6.2)	87 (4.6)	81 (6.1)	74 (6.7)
Triflu	0.05	0.999	(0.001)	83 (6.3)	84 (4.5)	76 (6.4)	70 (4.3)

LOD Limit of detection calculated as $3s_0$ where s_0 is the estimated standard deviation at zero concentration; R^2 (correlation coefficient of linear regression); SD standard deviation; SPE solid phase extraction; *Ben* bensulfuron methyl *Chlor* chlorsulfuron; *Etha* ethametsulfuron methyl; *Foram* foramsulfuron; *Halo* halosulfuron; *Meso* mesosulfuron methyl *Metsu* metsulfuron methyl; *Nico* nicosulfuron; *Oxa* oxasulfuron; *Primi* primisulfuron methyl; *Pro* prosulfuron *Rim* rimsulfuron; *Sulfom* sulfometuron methyl; *Sulfos* sulfosulfuron; *Thifen* thifensulfuron methyl; *Tria* triasulfuron; *Triflu* triflusulfuron methyl

Table 2 Accuracy and precision using pooled urine

Analyte	Accuracy		Precision (RSD)			
	Ave (%dev)	Ave (%dev)	WD		Total	
	3.0 (µg/L) n=50	10 (µg/L) n=50	3.0 (µg/L) n=50	10 (µg/L) n=50	3.0 (µg/L) n=50	10 (µg/L) n=50
Ben	2.6 (−13)	8.8 (−12)	6.3	4.3	11	6.6
Chlor	2.9 (−4.5)	9.6 (−4.4)	7.6	4.7	14	8.9
Etha	2.9 (−4.8)	9.6 (−3.9)	6.6	4.7	10	6.0
Foram	3.3 (11)	11 (12)	6.8	6.6	15	13
Halo	3.7 (22)	12 (19)	8.1	5.6	13	8.8
Meso	2.9 (−4.7)	9.7 (−3.0)	7.3	5.5	10	6.9
Metsu	2.8 (−5.8)	9.2 (−7.8)	5.6	4.0	11	6.8
Nico	4.4 (45)	14 (44)	6.9	6.4	18	13
Oxa	2.8 (−6.0)	9.3 (−7.1)	6.5	5.2	10	7.2
Primi	2.7 (−9.3)	9.1 (−9.0)	7.4	5.0	13	6.9
Pros	2.8 (−6.1)	9.4 (−5.8)	6.7	4.4	12	7.2
Rim	3.1 (2.0)	10 (1.6)	8.0	5.4	11	8.6
Sulfom	2.9 (−1.7)	9.7 (−2.7)	7.0	4.9	11	6.8
Sulfos	2.7 (−8.5)	9.4 (−6.0)	7.5	4.6	12	7.8
Thifen	3.3 (10)	11 (8.0)	6.1	5.4	11	7.2
Tria	2.8 (−6.0)	9.3 (−6.7)	7.0	4.1	11	5.9
Triflu	3.0 (−0.1)	9.8 (−2.1)	7.0	4.4	12	7.2

Accuracy average measured value compared to spiked concentration; *RSD* relative standard deviation; Ave average; (%dev) percent of average deviation from spiked concentration; *WD* within day; *Total* includes all sources of variability
Ben bensulfuron methyl; *Chlor* chlorsulfuron; *Etha* ethametsulfuron methyl; *Foram* foramsulfuron; *Halo* halosulfuron
Meso mesosulfuron methyl*; Metsu* metsulfuron methyl; *Nico* nicosulfuron; *Oxa* oxasulfuron; *Primi* primisulfuron methyl; *Pro* prosulfuron;
Rim rimsulfuron; *Sulfom* sulfometuron methyl; *Sulfos* sulfosulfuron
Thifen thifensulfuron methyl; *Tria* triasulfuron; *Triflu* triflusulfuron methyl

Table 3 Accuracy and precision using discrete urine samples

Analyte	Accuracy			Precision (RSD)								
	Ave (%dev)	Ave (%dev)	Ave (%dev)	BM			WM			Total		
	0.1 (µg/L) n=60	3.0 (µg/L) n=60	10 (µg/L) n=60	0.1 (µg/L) n=10	3.0 (µg/L) n=10	10 (µg/L) n=10	0.1 (µg/L) n=60	3.0 (µg/L) n=60	10 (µg/L) n=60	0.1 (µg/L) n=60	3.0 (µg/L) n=60	10 (µg/L) n=60
Ben	0.12 (16)	2.8 (−6.2)	10 (−0.2)	11	9.7	12	9.0	8.2	8.7	14	12	15
Chlor	0.13 (29)	3.2 (5.4)	11 (13)	13	11	13	11	7.7	8.6	16	13	15
Etha	0.09 (−6.0)	2.8 (−5.4)	10 (−2.2)	19	5.3	7	36	7.8	6.9	40	9.3	9.7
Foram	0.16 (60)	3.9 (30)	14 (41)	23	29	28	14	12	14	26	30	31
Halo	0.13 (26)	2.9 (−3.0)	12 (17)	14	16	20	14	15	14	19	21	24
Meso	0.10 (−2.1)	2.6 (−13)	10 (−1.5)	32	33	34	21	9.5	12	37	33	34
Metsu	0.11 (14)	2.8 (−6.3)	10 (0.1)	16	11	11	23	7.2	7.5	27	13	13
Nico	0.18 (77)	5.0 (67)	17 (69)	25	27	26	23	11	14	33	28	29
Oxa	0.12 (19)	2.9 (−1.8)	11 (10)	18	18	18	20	8.3	9.4	27	19	19
Primi	0.12 (18)	3.0 (−0.5)	11 (6.0)	13	12	14	15	7.9	8.1	19	14	16
Pros	0.11 (13)	3.2 (5.1)	11 (7.1)	15	12	15	14	11	10	20	16	18
Rim	0.11 (14)	2.3 (−23)	10 (3.9)	23	9.8	12	31[a]	10	9	38[a]	14	15
Sulfom	0.12 (19)	2.9 (−3.6)	10 (4.2)	12	5.6	6.8	35	8.4	6.9	37	10	9.5
Sulfos	0.11 (10)	2.7 (−10)	10 (0.3)	10	10	14	10	9.7	9	14	14	16
Thifen	0.13 (27)	3.1 (4.1)	11 (13)	28	30	29	22	7.6	8.5	34	30	29
Tria	0.11 (14)	2.9 (−2.1)	10 (4.9)	8.5	7.6	7.2	21	7.1	7.7	22	10	10
Triflu	0.11 (12)	2.7 (−11)	11 (14)	14	12	15	12	8.6	9.9	17	15	18

Accuracy average measured value compared to spiked concentration; *RSD* relative standard deviation; *Ave* average; (%dev) percent of average deviation from spiked concentration
WD within day; *Total* includes all sources of variability; *Ben* bensulfuron methyl; *Chlor* chlorsulfuron; *Etha* ethametsulfuron methyl; *Foram* foramsulfuron; *Halo* halosulfuron
Meso mesosulfuron methyl; *Metsu* metsulfuron methyl; *Nico* nicosulfuron; **Oxa** oxasulfuron; *Primi* primisulfuron methyl; *Pro* prosulfuron; *Rim* rimsulfuron; *Sulfom* sulfometuron methyl; *Sulfos* sulfosulfuron; *Thifen* thifensulfuron methyl; *Tria* triasulfuron; *Triflu* triflusulfuron methyl
[a]n=56 for these data

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